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(57) Abstract

The present invention provides a substantially pure or isolated oligodeoxynucleotide of at least about 10 nucleotides comprising a sequence represented by either the formula: 5' $N_1N_2N_3$T–CpG–W$N_4N_5N_6$ 3' wherein the central CpG motif is unmethylated, W is A or T, and N_1, N_2, N_3, N_4, N_5, and N_6 are any nucleotides, or the formula: 5' RY–CpG–RY 3' wherein the central CpG motif is unmethylated, R is A or G, and Y is C or T, as well as an oligodeoxynucleotide delivery complex and a pharmacological composition comprising the present inventive oligodeoxynucleotide, and a method of inducing an immune response by administering the present inventive oligodeoxynucleotide to a host.

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OLIGODEOXYNUCLEOTIDE AND ITS USE TO INDUCE AN IMMUNE RESPONSE

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to induction of an immune response using specific oligodeoxynucleotides (ODNs).

BACKGROUND OF THE INVENTION

DNA is a complex macromolecule whose immunological activities are influenced by its base composition and base modification, as well as helical orientation. Certain unusual DNA structures (e.g., Z-DNA) can induce significant antibody responses when administered to normal mice. In addition, bacterial DNA, as well as certain synthetic ODNs containing unmethylated CpG sequences can induce proliferation and immunoglobulin (Ig) production by murine B cells. Unmethylated CpG dinucleotides are more frequent in the genomes of bacteria and viruses than vertebrates. Recent studies suggest that immune recognition of these motifs may contribute to the host's innate immune response. D.M. Klinman et al., *CpG Motifs Present in Bacterial DNA Rapidly Induce Lymphocytes to Secrete Interleukin 6, Interleukin 12, and Interferon* γ, 93 Proc. Natl. Acad. Sci. USA 2879 (1996); A.-K. Yi et al., *Rapid Immune Activation by CpG Motifs in Bacterial DNA*, 157 J. Immun. 5394 (1996); Hua Liang et al., *Activation of Human B Cells by Phosphorothioate Oligodeoxynucleotides*, 98 J. Clin. Invest. 1119 (1996); A.M. Krieg et al., *CpG Motifs in Bacterial DNA Trigger Direct B-Cell Activation*, 374 Nature 546 (1995).

In mice, CpG DNA induces proliferation in almost all (>95%) of B cells and increases Ig secretion. This B-cell activation by CpG DNA is T-cell independent and antigen non-specific. In addition to its direct effects on B cells, CpG DNA also directly activates monocytes, macrophages, and dendritic cells to secrete a variety of cytokines. These cytokines stimulate natural killer (NK) cells to secrete γ-inteferon (IFN-γ) and have increased lytic activity. Examples of which can be found in International Patent Applications WO 95/26204, WO 96/02555, WO 98/11211, WO 98/18810, WO 98/37919, WO 98/40100, WO 98/52581, PCT/US98/047703, and PCT/US99/07335; U.S. Patent No. 5,663,153; and U.S. Patent Applications Serial

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Although bacterial DNA and certain ODNs can induce a murine immune response, little is known about the immunostimulatory capacity of these materials for the human immune system. Z.K. Ballas et al., *Induction of NK Activity in Murine and Human Cells by CpG Motifs in Oligodeoxynucleotides and Bacterial DNA*, 157 J. Immun. 1840 (1996). Differences in the responsiveness of human and murine B cells to certain stimuli render it impossible to extrapolate results obtained from mouse to man.

In view of the above, there exists a need for ODNs that induce an immune response in humans. In addition, there is a need for methods utilizing ODNs in the treatment of human diseases. The present invention provides such ODNs and methods of use. These and other advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a substantially pure or isolated ODN of at least about 10 nucleotides comprising a sequence represented by either the formula:

5' $N_1N_2N_3$T-CpG-W$N_4N_5N_6$ 3'

wherein the central CpG motif is unmethylated, W is A or T, and N_1, N_2, N_3, N_4, N_5, and N_6 are any nucleotides, or the formula:

5' RY-CpG-RY 3'

wherein the central CpG motif is unmethylated, R is A or G, and Y is C or T. The present invention also provides an ODN delivery complex and pharmacological composition comprising the present inventive ODN, as well as a method of inducing an immune response by administering the present inventive ODN to a host.

DETAILED DESCRIPTION OF THE INVENTION

Oligodeoxynucleotide

The present invention provides novel ODNs. These ODNs have at least about 10 nucleotides and comprise a sequence represented by either the formula:

5' $N_1N_2N_3$T-CpG-W$N_4N_5N_6$ 3'

wherein the central CpG motif is unmethylated, W is A or T, and N_1, N_2, N_3, N_4, N_5, and N_6 are any nucleotides, or the formula:

5' RY-CpG-RY 3'

wherein the central CpG motif is unmethylated, R is A or G, and Y is C or T. For example, the ODN can be selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 99.

Preferably, the ODN of the present invention is substantially pure or isolated. "Substantially pure" refers to an ODN that is substantially free of other materials, particularly other nucleic acids, proteins, lipids, carbohydrates, and other materials with which it may be naturally associated, while "isolated" refers to an ODN that is removed from its natural environment or state. Preferably, the ODN of the present invention consists of about 100 nucleotides or less (e.g., about 10-75 nucleotides). More preferably, the ODN consists of about 50 nucleotides or less (e.g., about 10-40 nucleotides). Even more preferably, the ODN consists of about 30 nucleotides or less (e.g., about 10-20 nucleotides). Most preferably the ODN consists of about 12 to about 16 nucleotides.

Any suitable modification can be used in the present invention to render the ODN resistant to degradation *in vivo* (e.g., via an exo or endonuclease). Preferably, the modification includes a phosphorothioate modification. The phosphorothioate modifications can occur at either termini, e.g., the last two or three 5' and/or 3' nucleotides can be liked with phosphorothioate bonds. The ODN also can be modified to contain a secondary structure (e.g., stem loop structure) such that it is resistant to degradation. Another modification that renders the ODN less susceptible

to degradation is the inclusion of nontraditional bases such as inosine and quesine, as well as acetyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine. Other modified nucleotides include nonionic DNA analogs, such as alkyl or aryl phosphonates (i.e., the charged phosphonate oxygen is replaced with an alkyl or aryl group, as set forth in U.S. Patent No. 4,469,863), phosphodiesters and alkylphosphotriesters (i.e., the charged oxygen moiety is alkylated, as set forth in U.S. Patent No. 5,023,243 and European Patent No. 0 092 574). ODNs containing a diol, such as tetraethyleneglycol or hexaethyleneglycol, at either or both termini, have also been shown to be more resistant to degradation.

Preferably, the ODNs inducing a humoral immune response, e.g., 5' $N_1N_2N_3$T-CpG-W$N_4N_5N_6$ 3', contain a phosphate backbone modification, and more preferably, the phosphate backbone modification is a phosphorothioate backbone modification (i.e., one of the non-bridging oxygens is replaced with sulfur, as set forth in International Patent Application WO 95/26204). For the ODNs inducing a cell-mediated immune response and containing a phosphodiester backbone, e.g., 5' RY-CpG-RY 3', the ODN preferably has been modified to prevent degradation.

Oligodeoxynucleotide Delivery Complex

The present inventive oligodeoxynucleotide delivery complex comprises the present inventive ODN and a targeting means. Any suitable targeting means can be used within the context of the present invention.

An ODN can be associated with (e.g., ionically or covalently bound to, or encapsulated within) a targeting means (e.g., a molecule that results in higher affinity binding to a target cell, such as a B cell). A variety of coupling or cross-linking agents can be used to form the delivery complex, such as protein A, carbodiamide, and N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP). Examples of ODN delivery complexes include ODNs associated with a sterol (e.g., cholesterol), a lipid (e.g., a cationic lipid, virosome or liposome), and a target cell specific binding agent (e.g., a ligand recognized by target cell specific receptor). Preferred complexes must be sufficiently stable *in vivo* to prevent significant uncoupling prior to internalization

by the target cell; however, these complexes can be cleavable under appropriate circumstances such that the ODN can be released in a functional form.

Pharmacological Composition

The present inventive pharmacological composition comprises the present inventive ODN and a pharmacologically acceptable carrier. Pharmacologically acceptable carriers (e.g., physiologically or pharmaceutically acceptable carriers) are well known in the art.

The present inventive pharmacological composition facilitates the use of the present inventive ODN, both *in vivo* and *ex vivo*. Such a composition can be suitable for delivery of the active ingredient to any suitable host, such as a patient for medical application, and can be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmacological compositions for use in accordance with the present invention can be formulated in a conventional manner using one or more pharmacologically (e.g., physiologically or pharmaceutically) acceptable carriers comprising excipients, as well as optional auxiliaries that facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Thus, for injection, the active ingredient can be formulated in aqueous solutions, preferably in physiologically compatible buffers. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For oral administration, the active ingredient can be combined with carriers suitable for inclusion into tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like. For administration by inhalation, the active ingredient is conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant. The active ingredient can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Such compositions can take such forms as suspensions, solutions or emulsions in oily or

aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Other pharmacological excipients are known in the art.

Method of Inducing an Immune Response

The present inventive method of inducing an immune response comprises administering the present inventive ODN to a host in order to induce an immune response in the host.

Administration of the present inventive ODN can be by any suitable method. For example, the ODN can be administered *in vivo* or *ex vivo*. Preferably, the ODN is administered *in vivo* to a mammal, particularly a human. Optionally, the ODN can be contained within or conjugated with a protein, hydrocarbon or lipid. Once this molecule is administered, the ODN sequence must be exposed on the surface to induce an immune response. The ODN can also be co-administered with a protein, hydrocarbon, or lipid. Co-administration can be such that the ODN is administered before, at substantially the same time as, or after the protein, hydrocarbon, or lipid. Preferably, the ODN is administered at substantially the same time as the protein, hydrocarbon, or lipid.

After administration of the novel ODNs, while not intending to be bound by any particular theory, it is thought that the ODNs initially act on antigen presenting cells (e.g., macrophages and dendritic cells). These cells then release cytokines, which activate natural killer (NK) cells. Either a cell-mediated or humoral immune response then occurs in the host.

The cell-mediated or local immune response is produced by T cells, which are able to detect the presence of invading pathogens through a recognition system referred to as the T-cell antigen receptor. Upon detection of an antigen, T cells direct the release of multiple T-cell cytokines, including IL-2, IL-3, IFN-γ, TNF-β, GM-CSF and high levels of TNF-α, and chemokines MIP-1α, MIP-1β, and RANTES. IL-2 is a T-cell growth factor that promotes the production of additional T cells sensitive to the particular antigen. This production constitutes a clone of the T cells. The sensitized T cells attach to cells containing the antigen. T cells carry out a variety of regulatory and defense functions and play a central role in immunologic responses. When stimulated to produce a cell-mediated immune response, some T cells respond by

acting as killer cells, killing the host's wn cells when these cells are infected r cancerous and therefore recognized as foreign. Some T cells respond by stimulating B cells, while other T cells respond by suppressing immune response. Preferably, if a cell-mediated immune response is induced, non-B cells are activated, more preferably, cytokines are produced, and most preferably, IFN-γ is produced.

The humoral or systemic immune response depends on the ability of the B cells to recognize specific antigens. The mechanism by which B cells recognize antigens is through specific receptors on the surface of the B cells. When an antigen attaches to the receptor site of a B cell, the B cell is stimulated to divide. The daughter cells become plasma cells that manufacture antibodies complementary to the attached antigen. Each plasma cell produces thousands of antibody molecules per minute, which are released into the bloodstream. Many B cells appear to be regulated by the helper T cells and suppressor T cells and produce various cytokines, e.g., IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, GM-CSF and low levels of TNF-α. Helper T cells stimulate B cells to produce antibodies against antigens, while suppressor T cells inhibit antibody production. Some B cells, however, are T-cell independent and require no stimulation by the T cells. Preferably, if a humoral immune response is induced, B cells are activated, more preferably, IL-6 is produced, and most preferably, antibodies are produced.

In addition, induction of one type of immune response may allow for immune regulation because up regulation of one type of immune response may down regulate the other type of immune response. This immune regulation allows for customizing or tailoring of the type of immune response when administering an ODN.

The present inventive method can be used to treat, prevent, or ameliorate any suitable allergic reaction in combination with any suitable anti-allergenic agent. An allergy, in the context of the present invention, refers to an acquired hypersensitivity to a substance (i.e., an allergen). Allergic conditions include eczema, allergic rhinitis or coryza, hay fever, bronchial asthma, uticaria (hives), food allergies, and other atopic conditions. The list of allergens is extensive and includes pollens, insect venoms, animal dander, dust, fungal spores, and drugs (e.g., penicillin). Examples of natural, animal, and plant allergens can be found in International Patent Application WO 98/18810. Preferably, the present inventive method is used to treat allergic

asthma. Suitable anti-allergenic agents include those substances given in treatment of the various allergic conditions described above, examples of which can be found in the Physicians' Desk Reference (1998).

The present inventive method can be used to treat any suitable cancer in combination with any suitable anti-cancer agent. Suitable cancers include cancers of the brain, lung (e.g., small cell and non-small cell), ovary, breast, prostate, and colon, as well as carcinomas and sarcomas. Preferably, the present inventive method is used to treat a solid tumor cancer. Suitable anti-cancer agents include those substances given in treatment of the various conditions described above, examples of which can be found in the Physicians' Desk Reference (1998).

The present inventive method can be used to improve the efficacy of any suitable vaccine. Suitable vaccines include those directed against Hepatitis A, B, and C, examples of which can be found in the Physicians' Desk Reference (1998), and DNA vaccines directed against HIV and malaria. *See generally* D. Klinman et al., *CpG Motifs as Immune Adjuvants*, 17 Vaccine 19 (1999); M.J. McCluskie and H.L. Davis, *CpG DNA is a Potent Enhancer of Systemic & Mucosal Immune Response Against Hepatitis B Surface Antigen with Intra-Nasal Administration to Mice*, 161 J. Immun. 4463 (1998).

The present inventive method can be used to treat, prevent, or ameliorate any suitable disease associated with the immune system. Preferred diseases associated with the immune system are autoimmune disorders and immune system deficiencies, e.g., lupus erythematosus, and autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. Immune system deficiencies include those diseases or disorders in which the immune system is not functioning at normal capacity, or in which it would be useful to boost the immune system response.

The present inventive method can be used with any suitable antisense therapy. Suitable antisense agents are those that bind either with DNA or RNA and block their function by inhibiting expression of the sequence to which the antisense agents are bound. *See generally* H. Lonnberg et al., *Towards Genomic Drug Therapy with Antisense Oligonucleotides*, 28 Ann. Med. 511 (1996); A. Alama et al., *Antisense Oligonucleotides as Therapeutic Agents*, 36 Pharmacol. Res. 171 (1997); K.J. Scanlon et al., *Oligonucleotide-Mediated Modulation of Mammalian Gene Expression*, 9

FASEB J. 1288 (1995); R. Oberbauer, *Not Non-Sense but Antisense -- Applications of Antisense Oligonucleotides in Different Fields of Medicine*, 109 Wien Klin Wochenschr 40 (1997).

The present inventive method can be used to treat, prevent, or ameliorate any suitable infection in combination with any suitable anti-infectious agent. Examples include francisella, schistosomiasis, tuberculosis, AIDS, malaria, and leishmania. Examples of suitable infectious viruses, bacteria, fungi, and other organisms (e.g., protists) can be found in International Patent Application WO 98/18810. Suitable anti-infectious agents include those substances given in treatment of the various conditions described elsewhere, examples of which can be found in the Physicians' Desk Reference (1998).

The present inventive method can be used to treat, prevent, or ameliorate the symptoms resulting from exposure to a bio-warfare agent. Suitable bio-warfare agents include those naturally occurring biological agents that have been specifically modified in the laboratory. Often, modification of these agents has altered them such that there is no known treatment. Examples include Ebola, Anthrax, and Listeria. In the course of ameliorating the symptoms after exposure, use of the present inventive ODNs may not cure the patient, but rather can extend the patient's life sufficiently such that some other treatment can then be applied.

The present invention is further described in the following examples. These examples are intended only to illustrate the invention and are not intended to limit the scope of the invention in any way.

EXAMPLES

Example 1

The following example demonstrates induction of an immune response by various ODNs. Induction was measured by production of the cytokines IL-6 and TNF-γ, and cell proliferation.

Human peripheral blood mononuclear cells (PBMC) were isolated, as described elsewhere (Z.K. Ballas et al., 85 J. Allergy Clin. Immunol. 453 (1990); Z.K. Ballas and W. Rasmussen, 45 J. Immunol. 1039 (1990); Z.K. Ballas and W. Rasmussen, 150 J. Immunol. 17 (1993)). ODNs were synthesized on a DNA

synthesizer (Applied Biosystems Inc., Foster City, CA), as described elsewhere (Beacage and Caruthers, *Deoxynucleoside Phosphoramidites – A New Class of Key Intermediates for Deoxypolynucleotide Synthesis*, 22 Tetrahedron Letters 1859 (1981)). In some ODNs, the normal DNA backbone phosphodiesterase linkages were replaced with phosphorothioate linkages, as described elsewhere (Agrawal et al., 94 Proc. Natl. Acad. Sci. USA 2620 (1997); Agrawal 14 TIB TECH 376 (1996)). To reduce degradation of the ODNs, those that did not have an entire phosphorothioate backbone contained phosphorothioate linkages at the 5' and 3' ends. Cells were incubated for approximately 72 hrs with the various ODNs. IL-6 and TNF-γ levels were determined by ELISA using anti-IL-6 and anti-TNF-γ antibodies, as described elsewhere (Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1989). Cell proliferation was determined by [^{3}H] thymidine incorporation, as described elsewhere (Liang et al., 98 J. Clin. Invest. at 1121).

IL-6 levels and cell proliferation are set forth in Table 1: Induction of a Humoral Immune Response *In Vitro*. These data demonstrate that a sequence containing 5' $N_1N_2N_3$T-CpG-W$N_4N_5N_6$ 3', wherein the central CpG motif is unmethylated, W is A or T, and N_1, N_2, N_3, N_4, N_5, and N_6 are any nucleotides, is desirable to induce a humoral immune response. In addition, maximum induction was observed for ODNs that contained a phosphorothioate backbone. IFN-γ levels and cell proliferation are set forth in Table 2: Induction of a Cell-Mediated Immune Response *In Vitro*. These data demonstrate that a sequence containing 5' RY-CpG-RY 3', wherein the central CpG motif is unmethylated, R is A or G and Y is C or T, is desirable to induce a cell-mediated immune response. Maximum induction occurred with ODNs containing phosphodiesterase linkages.

Table 1. Induction of a Humoral Immune Response *In Vitro*.

	IL-6 Levels (ELISA)	Cell Proliferation (^{3}H Thymidine Incorporation)
SEQ ID NO: 1	65	52
SEQ ID NO: 2	85	44
SEQ ID NO: 3	54	50

	IL-6 Levels (ELISA)	Cell Proliferation (^{3}H Thymidine Incorporation)
SEQ ID NO: 4	48	61
SEQ ID NO: 5	42	100
SEQ ID NO: 6	55	23
SEQ ID NO: 7	35	69
SEQ ID NO: 8	28	38
SEQ ID NO: 9	41	20
SEQ ID NO: 10	42	16
SEQ ID NO: 11	33	77
SEQ ID NO: 12	25	13
SEQ ID NO: 13	28	13
SEQ ID NO: 14	35	67
SEQ ID NO: 15	28	54
SEQ ID NO: 16	39	50
SEQ ID NO: 17	50	32
SEQ ID NO: 18	26	1
SEQ ID NO: 19	12	2
SEQ ID NO: 20	55	92
SEQ ID NO: 21	53	26
SEQ ID NO: 22	8	2
SEQ ID NO: 23	12	1
SEQ ID NO: 24	14	0
SEQ ID NO: 25	30	42
SEQ ID NO: 26	43	60
SEQ ID NO: 27	17	15
SEQ ID NO: 28	14	0
SEQ ID NO: 29	10	1
SEQ ID NO: 30	28	23
SEQ ID NO: 31	16	17

Table 2. Induction of a Cell-Mediated Immune Response *In Vitro*.

	IFN-γ Levels (ELISA)	Cell Proliferation (^{3}H Thymidine Incorporation)
SEQ ID NO: 32	78	1
SEQ ID NO: 33	100	2
SEQ ID NO: 34	73	2
SEQ ID NO: 35	88	4

	IFN-γ Levels (ELISA)	Cell Proliferation (^{3}H Thymidine Incorporation)
SEQ ID NO: 36	81	5
SEQ ID NO: 37	45	4
SEQ ID NO: 38	78	0
SEQ ID NO: 39	33	5
SEQ ID NO: 40	68	2
SEQ ID NO: 41	54	2
SEQ ID NO: 42	54	1
SEQ ID NO: 43	74	4
SEQ ID NO: 44	53	4
SEQ ID NO: 45	32	9
SEQ ID NO: 46	24	1
SEQ ID NO: 47	23	8
SEQ ID NO: 48	22	25
SEQ ID NO: 49	34	26
SEQ ID NO: 50	36	8
SEQ ID NO: 51	24	17
SEQ ID NO: 52	21	9
SEQ ID NO: 53	19	2
SEQ ID NO: 54	12	8
SEQ ID NO: 55	15	5
SEQ ID NO: 56	22	6
SEQ ID NO: 57	18	3
SEQ ID NO: 58	18	6
SEQ ID NO: 59	12	21
SEQ ID NO: 60	13	4
SEQ ID NO: 61	--	2
SEQ ID NO: 62	12	23
SEQ ID NO: 63	16	1
SEQ ID NO: 64	16	4
SEQ ID NO: 65	19	2
SEQ ID NO: 66	16	4
SEQ ID NO: 67	14	2
SEQ ID NO: 68	13	1
SEQ ID NO: 69	12	2
SEQ ID NO: 70	19	2
SEQ ID NO: 71	13	1
SEQ ID NO: 72	14	46
SEQ ID NO: 73	--	4
SEQ ID NO: 74	16	1
SEQ ID NO: 75	24	1

	IFN-γ Levels (ELISA)	Cell Proliferation (^{3}H Thymidine Incorporation)
SEQ ID NO: 76	13	1
SEQ ID NO: 77	12	1
SEQ ID NO: 78	13	1
SEQ ID NO: 79	13	1
SEQ ID NO: 80	12	1
SEQ ID NO: 81	14	20
SEQ ID NO: 82	14	43
SEQ ID NO: 83	14	1
SEQ ID NO: 84	12	1
SEQ ID NO: 85	15	2
SEQ ID NO: 86	13	1
SEQ ID NO: 87	12	0
SEQ ID NO: 88	–	3
SEQ ID NO: 89	15	1
SEQ ID NO: 90	18	2
SEQ ID NO: 91	13	2
SEQ ID NO: 92	12	1
SEQ ID NO: 93	14	2
SEQ ID NO: 94	14	1
SEQ ID NO: 95	44	3
SEQ ID NO: 96	24	1
SEQ ID NO: 97	21	6
SEQ ID NO: 98	36	38
SEQ ID NO: 99	21	26

The foregoing data demonstrates the induction of an immune response in human cells, as exemplified by PBMC, and as measured by the production of the cytokines IFN-γ and IL-6, and cell proliferation, occurs upon the administration of various ODNs.

Example 2

The following example demonstrates induction of an immune response *ex vivo* by various ODNs. Induction was measured by production of the cytokine IL-6.

A human B cell line (RPMI 8226) was maintained according to the manufacturers recommendations. ODNs were synthesized as described in Example 1. In some ODNs, the normal DNA phosphodiesterase linkages were replaced with

phosphorothioate linkages, as described in Example 1. To reduce degradation of the ODNs, those that did not have an entire phosphorothioate backbone contained phosphorothioate linkages at the ends. The cells were incubated with various ODNs for 14 hrs. IL-6 production was determined by ELISA using anti-IL-6 antibodies, as described in Example 1.

IL-6 levels are set forth in Table 3: Induction of a Humoral Immune Response *Ex Vivo*. These data confirm that a sequence containing 5' $N_1N_2N_3$T-CpG-W$N_4N_5N_6$ 3', which are linked by phosphorothioate bonds and wherein the central CpG motif is unmethylated, W is A or T, and N_1, N_2, N_3, N_4, N_5, and N_6 are any nucleotides, is desirable to induce a humoral immune response.

Table 3. Induction of a Humoral Immune Response *Ex Vivo*.

	IL-6 Levels (ELISA)
SEQ ID NO: 1	100
SEQ ID NO: 2	89
SEQ ID NO: 3	85
SEQ ID NO: 4	82
SEQ ID NO: 5	82
SEQ ID NO: 6	78
SEQ ID NO: 7	78
SEQ ID NO: 8	78
SEQ ID NO: 9	73
SEQ ID NO: 10	65
SEQ ID NO: 11	62
SEQ ID NO: 12	58
SEQ ID NO: 13	57
SEQ ID NO: 14	56
SEQ ID NO: 15	50
SEQ ID NO: 16	48
SEQ ID NO: 17	47
SEQ ID NO: 18	45
SEQ ID NO: 19	40
SEQ ID NO: 20	39
SEQ ID NO: 21	33
SEQ ID NO: 22	25
SEQ ID NO: 23	23

	IL-6 Levels (ELISA)
SEQ ID NO: 24	21
SEQ ID NO: 25	18
SEQ ID NO: 26	17
SEQ ID NO: 27	17
SEQ ID NO: 28	16
SEQ ID NO: 29	16
SEQ ID NO: 30	13
SEQ ID NO: 31	13

The foregoing data demonstrates the induction of an immune response in human cells, as exemplified by the human B cell line RPMI 8226, and as measured by production of the cytokine IL-6, occurs upon administration of various ODNs.

The following table lists additional ODNs which fall within the scope of the present invention.

Table 4:

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SEQ ID NO: 138
SEQ ID NO: 139
SEQ ID NO: 140
SEQ ID NO: 141
SEQ ID NO: 142
SEQ ID NO: 143

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WHAT IS CLAIMED IS:

1. A substantially pure or isolated oligodeoxynucleotide of at least about 10 nucleotides comprising a sequence represented by the following formula:

5' $N_1N_2N_3$T-CpG-W$N_4N_5N_6$ 3'

wherein the central CpG motif is unmethylated, W is A or T, and N_1, N_2, N_3, N_4, N_5, and N_6 are any nucleotides.

2. A substantially pure or isolated oligodeoxynucleotide of at least about 10 nucleotides comprising a sequence represented by the following formula:

5' RY-CpG-RY 3'

wherein the central CpG motif is unmethylated, R is A or G and Y is C or T.

3. The oligodeoxynucleotide of claim 2, wherein the sequences on the 5' side of the CpG sequences form a palindrome with the sequences on the 3' side of the CpG sequence.

4. The oligodeoxynucleotide of any of claims 1-3, wherein the oligodeoxynucleotide is selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 143.

5. The oligodeoxynucleotide of any of claims 1-4, wherein the oligodeoxynucleotide is modified to prevent degradation.

6. The oligodeoxynucleotide of any of claims 1-5, wherein the oligodeoxynucleotide has a phosphate backbone modification.

7. The oligodeoxynucleotide of claim 6, wherein the phosphate backbone modification is a phosphorothioate backbone modification.

8. The oligodeoxynucleotide of any of claims 1-7, wherein the oligodeoxynucleotide comprises about 100 nucleotides or less.

9. The oligodeoxynucleotide claim 8, wherein the oligodeoxynucleotide comprises about 50 nucleotides or less.

10. The oligodeoxynucleotide of claim 9, wherein the oligodeoxynucleotide comprises about 30 nucleotides or less.

11. The oligodeoxynucleotide of claim 10, wherein the oligodeoxynucleotide comprises about 12-16 nucleotides.

12. An oligodeoxynucleotide delivery complex comprising the oligodeoxynucleotide of any of claims 1-11 and a targeting means.

13. The oligodeoxynucleotide delivery complex of claim 12, wherein the targeting means is selected from the group consisting of cholesterol, virosome, liposome, lipid, and a target cell specific binding agent.

14. A pharmacological composition comprising the oligodeoxynucleotide of any of claims 1-11 and a pharmacologically acceptable carrier.

15. A method of inducing an immune response in a host comprising administering to a host an oligodeoxynucleotide of any of claims 1-11.

16. The method of claim 15, wherein the immune response induced is a cell-mediated immune response.

17. The method of claim 15 or 16, wherein the oligodeoxynucleotide activates non-B cells in the host.

18. The method of any of claims 15-17, wherein the oligodeoxynucleotide induces cytokine production in the host.

19. The method of claim 18, wherein the cytokine is IFN-γ.

20. The method of claim 15, wherein the immune response induced is a humoral immune response.

21. The method of claim 15 or 20, wherein the oligodeoxynucleotide activates B cells in the host.

22. The method of any of claims 15, 20, or 21, wherein the oligodeoxynucleotide induces IL-6 production in the host.

23. The method of any of claims 15, 20-22, wherein the oligodeoxynucleotide induces antibody production in the host.

24. The method of any of claims 15-23, wherein the induction of an immune response is used to treat, prevent, or ameliorate an allergic reaction, and the oligodeoxynucleotide is administered either alone or in combination with an anti-allergenic agent.

25. The method of claim 24, wherein the allergic reaction is asthmatic.

26. The method of any of claims 15-23, wherein the induction of an immune response is used to treat cancer, and the oligodeoxynucleotide is administered either alone or in combination with an anti-cancer agent.

27. The method of claim 26, wherein the cancer is a solid tumor cancer.

28. The method of any of claims 15-23, wherein the induction of an immune response is used to improve the efficacy of a vaccine, and the oligodeoxynucleotide is administered either alone or in combination with a vaccine.

29. The method of any of claims 15-23, wherein the induction of an immune response is used to treat, prevent or ameliorate a disease associated with the immune system.

30. The method of claim 29, wherein the disease associated with the immune system is an autoimmune disorder.

31. The method of claim 29, wherein the disease associated with the immune system is an immune system deficiency.

32. The method of any of claims 15-23, wherein the induction of an immune response is used in antisense therapy, and the oligodeoxynucleotide is administered either alone or in combination with an antisense agent.

33. The method of any of claims 15-23, wherein the induction of an immune response is used to treat, prevent, or ameliorate an infection, and the oligodeoxynucleotide is administered either alone or in combination with an anti-infectious agent.

34. The method of any of claims 15-23, wherein the induction of an immune response is used to treat, prevent, or ameliorate the symptoms resulting from exposure to a bio-warfare agent.

35. The method of any of claims 15-34, wherein the method further comprises:

(a) administering the oligodeoxynucleotide to lymphocytes *ex vivo*, thereby producing activated lymphocytes, and

(b) administering the activated lymphocytes obtained in step (a) to the host.

36. The method of any of claims 15-34, wherein the host is a human.

SEQUENCE LISTING

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Verthelyi, Daniela
Ishii, Ken

<120> OLIGODEOXYNUCLEOTIDE AND ITS USE TO INDUCE AN IMMUNE RESPONSE

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Published:
— *With international search report.*

(88) Date of publication of the international search report:
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/61151 A3

(54) Title: OLIGODEOXYNUCLEOTIDE AND ITS USE TO INDUCE AN IMMUNE RESPONSE

(57) Abstract: The present invention provides a substantially pure or isolated oligodeoxynucleotide of at least about 10 nucleotides comprising a sequence represented by either the formula: 5' $N_1N_2N_3$T-CpG-W$N_4N_5N_6$ 3' wherein the central CpG motif is unmethylated, W is A or T, and N_1, N_2, N_3, N_4, N_5, and N_6 are any nucleotides, or the formula: 5' RY-CpG-RY 3' wherein the central CpG motif is unmethylated, R is A or G, and Y is C or T, as well as an oligodeoxynucleotide delivery complex and a pharmacological composition comprising the present inventive oligodeoxynucleotide, and a method of inducing an immune response by administering the present inventive oligodeoxynucleotide to a host.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 00/09839

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/70 A61K39/39 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 37919 A (UNIV IOWA RES FOUND) 3 September 1998 (1998-09-03) tables 5-7	1-36
X	WO 96 02555 A (UNIV IOWA RES FOUND) 1 February 1996 (1996-02-01) in particular sequences 8,10,11,14,15 the whole document	1-36
X	WO 95 26204 A (ISIS PHARMACEUTICALS INC) 5 October 1995 (1995-10-05) in particular sequence 2	1,4-36
X	EP 0 468 520 A (MITSUI TOATSU CHEMICALS) 29 January 1992 (1992-01-29) in particular sequences 16,37,42,48-50,53,56 claims 10,16	1-36

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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 December 2000	10.01.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	Bardili, W

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 55495 A (DYNAVAX TECHNOLOGIES CORP ;DINA DINO (US); ROMAN MARK (US); SCHWAR) 10 December 1998 (1998-12-10) in particular sequences 1,2,4,5,11,15,17,19 ---	1,4-36
X	WO 98 18810 A (UNIV IOWA RES FOUND ;KLINE JOEL N (US); KRIEG ARTHUR M (US)) 7 May 1998 (1998-05-07) claims 4-9,23,26,29 ---	1-36
X	WO 98 52581 A (WU TONG ;DAVIS HEATHER L (CA); OTTAWA CIVIC HOSPITAL LOEB RES (CA)) 26 November 1998 (1998-11-26) in particular claims 7,8,10-12,14,50,52-54,56,66,68-70,72,100, 102-104,106 ---	1,4-36
P,X	WO 99 56755 A (OTTAWA CIVIC LOEB RESEARCH INS ;UNIV IOWA RES FOUND (US); US OF AM) 11 November 1999 (1999-11-11) the whole document ---	1-36
P,X	WO 99 51259 A (UNIV IOWA RES FOUND) 14 October 1999 (1999-10-14) in particular sequences on pages 52-54 the whole document ---	1-36
P,X	WO 99 58118 A (CPG IMMUNOPHARMACEUTICALS GMBH ;CPG IMMUNOPHARMACEUTICALS INC (US)) 18 November 1999 (1999-11-18) in particular sequences on pages 65-67 the whole document ---	1-36
P,X	WO 99 61056 A (LOEB HEALTH RESEARCH INST AT T ;CPG IMMUNOPHARMACEUTICALS INC (US)) 2 December 1999 (1999-12-02) in particular sequences on pages 23-25 claim 18 ---	1-36
P,X	WO 99 62923 A (DYNAVAX TECHNOLOGIES CORP ;SCHWARTZ DAVID (US)) 9 December 1999 (1999-12-09) table 1 ---	1,4-36
X	WO 93 17115 A (BIOTECHNOLOG FORSCHUNG GMBH) 2 September 1993 (1993-09-02) claim 16 ---	2
X	EP 0 855 184 A (HEEG KLAUS PROF DR ;LIPFORD GRAYSON B DR (DE); WAGNER HERMANN PROF) 29 July 1998 (1998-07-29) see sequence no. 3 --- -/--	2

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INTERNATIONAL SEARCH REPORT

Inter ·nal Application No
PCT/US 00/09839

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 18231 A (SECR DEFENCE BRIT ;TITBALL RICHARD WILLIAM (GB); WILLIAMSON ETHEL) 6 July 1995 (1995-07-06) see sequence no. 2 ---	2
X	EP 0 572 735 A (AMOCO CORP) 8 December 1993 (1993-12-08) table 1 -----	2

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/09839

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claims may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, a meaningful search over the whole breadth of the claim(s) is impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1,4-36 (partially)

   Oligodeoxynucleotides comprising CpG mofifs embedded in a sequence as depicted in claim 1, compositons comprising said oligodeoxynucleotides and their use to induce an immune response in a host.

2. Claims: 2,3,4-36 (partially)

   Oligodeoxynucleotides comprising a CpG motif embedded in a sequence as depicted in claim 2, compositions comprising said oligodeoxynucleotides and their use to induce an immune response in a host

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/US 00/09839

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9837919 A	03-09-1998	AU 6667498 A	18-09-1998
		EP 1039935 A	04-10-2000
WO 9602555 A	01-02-1996	AU 713040 B	18-11-1999
		AU 1912795 A	16-02-1996
		CA 2194761 A	01-02-1996
		EP 0772619 A	14-05-1997
		JP 10506265 T	23-06-1998
		US 6008200 A	28-12-1999
WO 9526204 A	05-10-1995	US 5663153 A	02-09-1997
		US 5723335 A	03-03-1998
EP 0468520 A	29-01-1992	JP 4352724 A	07-12-1992
WO 9855495 A	10-12-1998	AU 7811398 A	21-12-1998
		AU 7817898 A	21-12-1998
		EP 1003850 A	31-05-2000
		EP 0986572 A	22-03-2000
		WO 9855609 A	10-12-1998
WO 9818810 A	07-05-1998	AU 5242498 A	22-05-1998
		CN 1235609 A	17-11-1999
		EP 0948510 A	13-10-1999
WO 9852581 A	26-11-1998	AU 7690898 A	11-12-1998
		EP 1003531 A	31-05-2000
WO 9956755 A	11-11-1999	AU 3884199 A	23-11-1999
WO 9951259 A	14-10-1999	AU 3467899 A	25-10-1999
WO 9958118 A	18-11-1999	AU 4528899 A	29-11-1999
WO 9961056 A	02-12-1999	AU 4197799 A	13-12-1999
WO 9962923 A	09-12-1999	AU 4419499 A	20-12-1999
WO 9317115 A	02-09-1993	AU 3628193 A	13-09-1993
EP 0855184 A	29-07-1998	AU 724325 B	14-09-2000
		AU 6293498 A	18-08-1998
		WO 9832462 A	30-07-1998
		EP 0971736 A	19-01-2000
WO 9518231 A	06-07-1995	AU 1322295 A	17-07-1995
		CA 2179639 A	06-07-1995
		EP 0741786 A	13-11-1996
		JP 9507028 T	15-07-1997
EP 0572735 A	08-12-1993	DE 69227678 D	07-01-1999
		DE 69227678 T	27-05-1999